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The Fate of Intrapleurally Injected Bone Marrow-Derived Stem Cells in Mice with Pleural Mesothelioma

PRINCIPAL INVESTIGATOR:

Jonathan M. Horowitz, PhD

CONTRACTING ORGANIZATION:

North Carolina State University
Raleigh, NC 27695-7003

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14. ABSTRACT
This research contract focused on the utility of genetically-engineered bone marrow stem cells (BMSCs) as potential therapeutics against malignant mesothelioma (MM). Our over-arching goal was to determine whether fluorescently-tagged murine BMSCs introduced into a mouse model for MM would home to and infiltrate diseased and normal tissues of interest. Protocols for the isolation, amplification, characterization, and intrapleural injection of fluorescently-tagged BMSCs were developed and optimized where possible. A number of significant technical challenges were encountered, including limiting numbers of viable BMSCs, contamination of BMSC cultures, and histocompatibility issues following intrapleural injection. A fully syngeneic approach was adopted to ameliorate some of these concerns, and injected animals were characterized by indirect immunofluorescence for the presence of BMSCs as well as histopathological changes. Unfortunately, BMSC injection had little or no significant gross effect on mesothelioma progression or pathology.

15. SUBJECT TERMS- malignant mesothelioma, bone marrow stem cells

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Introduction

Epidemiological evidence indicates that current and former U.S. military personnel are at increased risk of developing malignant mesothelioma (MM)[1]. Up to 30 per cent of all mesothelioma cases have been correlated with prior military service, with former members of the U.S. Navy at particularly elevated risk. Inadvertent asbestos exposure is believed to be the primary cause of these malignancies in virtually all instances. Despite the introduction of aggressive therapeutic treatment strategies the prognosis for MM patients is extremely poor, underscoring the need for novel, efficacious therapies.

Bone marrow-derived stem cells (BMSCs) have been shown to home to the tumor microenvironment, often helping to support the growth of malignant cells[2]. It is as yet uncertain whether tumor-infiltrating BMSCs fuse with cells within the tumor microenvironment, differentiate into tumor-associated cells or remain in a largely undifferentiated state. Given the capacity of BMSCs to home to sites of tumor cell deposition, the hypothesis that provoked the current study is that the intrapleural injection of genetically-engineered BMSCs will integrate within MM tissue and affect tumor growth. As such, these cells could be useful as tools to facilitate tumor imaging or to carry novel cargoes, including cancer therapeutics.

Body

In contrast to tumors surrounded by neighboring normal tissues, a large proportion of MM tissue is exposed within the body cavity. This relatively exposed disposition renders intracavitary delivery of anticancer agents an attractive therapeutic alternative as it would be anticipated to limit side effects associated with systemic delivery. Indeed, it has been reported that BMSCs injected into the body cavity migrate to and colonize in visceral organs and bone marrow[3]. However, the fate of intracavitary injected BMSCs within growing MM has not been investigated. This relatively circumscribed research project tested our hypothesis with two specific aims. First, we proposed to determine the effects of intrapleurally-injected BMSCs in an *in vivo* mouse model of human MM. Second, we proposed to determine the distribution of stromal cells derived from BMSCs in MM tissues. Two sets of mice (donors and recipients) were employed for these studies. The recipients are homozygous for "floxed" Nrf2 and Arf genes, and develop MM within six months subsequent to the intrapleural injection of Adenovirus vectors that express Cre recombinase (Cre recombinase induces the conditional inactivation of Nrf2 and Arf via Cre-mediated recombination)[4]. The donors are transgenic animals (C57BL/6-Tg(CAG-EGFP)131Osb/LeySopJ) in which green fluorescent protein (GFP) is expressed constitutively in all cells, including BMSCs. We anticipated that the fluorescent tag carried by these cells would facilitate their identification within recipient tissues.

The first seven months of effort on this project was consumed by preparatory experiments to determine the fate of BMSCs injected intrapleurally. These preparatory steps included (i) IACUC protocol approval, (ii) obtaining and breeding transgenic and conditional "knock-out" animals to be used as BMSCs donors, (iii) establishing protocols for the isolation, culture and characterization of murine BMSCs, (iv) establishing protocols for the intrapleural injection and characterization of BMSCs, (v) obtaining Adenovirus stocks encoding Cre recombinase (Univ. of Iowa Gene Transfer Vector Core lab) and (vi) preparation of fixed tissues for evaluation by indirect immunofluorescence. In so doing, we made significant progress on Tasks 1-3 of Specific Aim #1 and completed all three milestones. With this said, we encountered a series of significant technical hurdles that ultimately delayed further progress and prevented us from completing Specific Aim #2. As is common with genetically-engineered animals, we found that

the strains employed bred inefficiently and this limited the number of experiments that we were able to perform. We also had difficulty generating MM efficiently in genetically engineered animals, and thus spent considerable time optimizing our intrapleural injections of recombinant Adenoviruses. The development of sterile protocols to harvest, amplify, and culture sufficient quantities of BMSCs also proved to be challenging. Indeed, many BMSCs appeared to perish in vitro via apoptosis and we altered a number of culture conditions (*e.g.*, oxygen tension) to identify a protocol that resulted in the reliable rescue of explanted cells. Finally, as predicted in our original application, histocompatibility issues proved to be a hindrance and we had to adopt a fully syngeneic approach in the final months of our project period. Adoption of a syngeneic approach presented additional challenges, as we spent considerable time optimizing the delivery of a recombinant lentivirus (engineered to carry GFP) into BMSCs and using flow cytometry to select GFP-positive derivatives for analysis *in vivo*. As a consequence of these technical issues, we were not able to conclude whether or not our hypothesis had merit.

Key Research Accomplishments

- Developed protocols for the isolation, culture and characterization of murine BMSCs
- Developed protocols for the intrapleural injection and characterization of BMSCs
- Developed protocols for a fully syngeneic approach to intrapleural BMSC injection

Reportable Outcomes

None.

Conclusion

Although we were not able to obtain evidence that supports or negates our hypothesis, we remain encouraged that the approach employed could be a viable treatment alternative for patients with MM. BMSCs were harvested, cultivated, and injected intrapleurally into recipients and we were able to track their distribution *in vivo* within the intrapleural cavity and peripheral tissues. Additional experiments will be required to take advantage of what we learned during this year-long project and in so doing address (i) the degree to which BMSCs infiltrate MM and (ii) the disposition of these cells post-integration within the tumor microenvironment.

References

1. Butnor, K. J., Sharma, A., Sporn, T. A., and Roggli, V. L. Malignant Mesothelioma and Occupational Exposure to Asbestos: An Analysis of 1445 Cases. *Ann Occup Hyg*, 46: 150-153, 2002.
2. Li, H., Fan, X., and Houghton, J. Tumor microenvironment: the role of the tumor stroma in cancer. *J Cell Biochem*, 101: 805-815, 2007.
3. Chamberlain, J., Yamagami, T., Colletti, E., Theise, N. D., Desai, J., Frias, A., Pixley, J., Zanjani, E. D., Porada, C. D., and Almeida-Porada, G. Efficient generation of human hepatocytes by the intrahepatic delivery of clonal human mesenchymal stem cells in fetal sheep. *Hepatology*, 46: 1935-1945, 2007.
4. Jongsma, J., van Montfort, E., Vooijs, M., Zevenhoven, J., Krimpenfort, P., van der Valk, M., van de Vijver, M., Berns, A. *Cancer Cell*, 13: 261-271, 2008.

Appendices None.